Amulic, B., Moxon, C. A. and Cunnington, A. J. (2020) A more granular view of neutrophils in malaria. Trends in Parasitology, 36(6), pp. 501-503.

There may be differences between this version and the published version. You are advised to consult the publisher's version if you wish to cite from it.

http://eprints.gla.ac.uk/211952/

Deposited on: 3 July 2020

Enlighten – Research publications by members of the University of Glasgow
http://eprints.gla.ac.uk

A more granular view of neutrophils in malaria

Borko Amulic[1], Christopher Moxon[2] and Aubrey Cunnington[3]

[1] Cellular and Molecular Medicine, University of Bristol, UK.

[2] Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity and Inflammation, College of Medical Veterinary & Life Sciences, University of Glasgow, UK

[3] Department of Infectious Disease, Faculty of Medicine, Imperial College London, UK

[*] borko.amulic@bristol.ac.uk (B. Amulic)

Keywords: malaria, neutrophils, innate immunity

Abstract

Neutrophils are abundant innate immune cells with crucial roles in immunity and vascular inflammation. Recent evidence indicates that neutrophils have a dual role in malaria, contributing to both pathogenesis and control of *Plasmodium*. We discuss emerging mechanisms behind these opposing functions and identify key outstanding questions.

Hiding in plain sight

Neutrophils, also known as polymorphonuclear granulocytes, account for up to 70% of all blood leukocytes. They are essential for defense against pathogens; a severely reduced neutrophil count (neutropenia) results in elevated susceptibility to bacterial and fungal infections. Despite their importance in immunity, until recently neutrophils have been relatively neglected in studies of malaria. This is surprising considering that the asexual, disease-causing forms of *Plasmodium* also circulate in blood, where they invade erythrocytes and achieve high densities that match or exceed those of their granular neighbours. Moreover, neutrophil numbers often (but not always) rise in malaria patients [1], in contrast to circulating lymphocytes, which decrease during *P. falciparum* infections.

Why this relative dearth of information on neutrophil responses in malaria? Neutrophil research is impeded by technical challenges, including their short life span (12-hour half-life in culture) and lack of viable cell preservation methods. Furthermore, neutrophils do not separate with PBMCs in common white blood cell isolation protocols. Thus, work with neutrophils is particularly unsuited to the challenges of field work in malaria endemic countries. Common mouse models of malaria pathogenesis, on the other hand, are heavily T-cell driven, which may obscure the neutrophil contribution.

In spite of these challenges, there is increasing evidence that neutrophils might play important roles in malaria. Recent studies in humans and mouse models have indicated that different neutrophil defense mechanisms might determine the balance between pathogenesis and protection [2-4], and have highlighted the need for a more granular view of neutrophil responses in malaria.

Too much of a good thing?

Neutrophil granules contain potent antimicrobial proteins that efficiently kill pathogens (Box 1). However, these molecules are also inflammatory and cytotoxic to host tissues. Their indiscriminate release can harm the host, particularly when this occurs systemically. Yet recent evidence indicates that this is precisely what occurs in severe malaria, with frequent detection of elevated plasma levels of granule proteins [5] and the neutrophil chemokine IL-8 [6]. Neutrophil activation is also reflected in a blood transcriptional signature showing neutrophil transcripts to be associated with severe disease [7]. NETs are selectively detected in the neurovasculature of fatal pediatric cerebral malaria patients (using retinal tissue), co-localized with iRBCs, suggesting a role in sequestration-driven pathology. In the *P. chabaudi* mouse model, NETs are triggered by extracellular heme (released as a result of RBC destruction) and cleavage of NETs by plasma DNAse 1 releases immunostimulatory molecules that drive endothelial activation and facilitate organ sequestration of iRBCs [2]. NET release, and possibly degranulation, are therefore implicated in key pathogenic processes in severe malaria.

Protective instincts

Whilst NETosis and degranulation are implicated in the immunopathology of malaria, these defense mechanisms may not be exclusively harmful. Amongst the myriad of granule proteins, some may have antiparasitic properties (although identifying them is a challenge), and it remains to be determined whether the extensive degranulation seen in severe malaria is an appropriate or dysfunctional response to very high

parasite load. By using a mathematical modelling approach to estimate the extent of parasite growth inhibition in naturally occurring malaria in Gambian children, several neutrophil proteins were identified as correlates of protection, distinct from those associated with severity [8]. Inter-individual variation causing higher expression of cathepsin G and matrix metallopeptidase 9 (*MMP9*) enhanced the overall antiparasitic response. *In vitro* experiments suggested that MMP-9 acted as a classical antimicrobial protein, targeting parasites directly, but surprisingly cathepsin G did not. Rather than acting against the parasites, cathepsin G appeared to mediate defense by cleaving the red cell surface molecules necessary for parasite invasion, essentially removing the handle from the door [8]. Interestingly, whilst the majority of receptors for *P. falciparum* invasion receptors were cleaved in a dose-dependent manner, the lack of cleavage of CD55 (decay accelerating factor, which is also needed to prevent red cell destruction by complement) suggests a degree of specificity. Further work will be needed to confirm that these mechanisms are truly relevant *in vivo*, in the presence of naturally occurring protease inhibitors. We believe it is likely that other neutrophil proteases could produce similar effects and may also have antimalarial activity, but discriminating their individual contributions to control of parasite load will be a big task.

It remains unclear to what extent phagocytosis by neutrophils controls *Plasmodium* proliferation. *In vitro*, neutrophils can phagocytose free merozoites, gametocytes or entire infected red blood cells (iRBCs) [1]. In patient blood smears, neutrophil internalization of parasites can sometimes be seen [9], although this is rarely the case and has not been systematically analyzed (more data is available for hemozoin). More relevant to suppression of *Plasmodium* growth may be antibody-dependent phagocytosis, which requires immunoglobulin opsonization of parasites. This mechanism relies on infection-induced antibodies and complement [10] and therefore

wouldn't be expected in primary infections or acute infection of naïve mice. Phagocytic killing of *Plasmodium* may thus depend on chronicity and previous exposure.

Not all made equal

The variation in granule protein expression represents a subtle heterogeneity in neutrophils between individuals, but greater heterogeneity of neutrophil function within individuals has also been shown. During malaria, a population of neutrophils with reduced oxidative burst capacity is seen in the circulation, and persists for up to 8 weeks after infection [11], far longer than the short lifespan of individual neutrophils. This subset of neutrophils was evident in bone marrow in malaria infected mice, and appeared to be dependent on the induction of heme oxygenase-1 in granulocyte progenitors, and increased mobilization of these neutrophils into the circulation as a consequence of malaria-induced hemolysis. The production of neutrophils with reduced capacity to generate reactive oxygen species may be an adaptive response to malaria, because cell-free heme is a potent stimulus for oxidative tissue damage and organ pathology. Other malaria-induced changes in neutrophil function have also been observed in humans and mice, including the above mentioned enhanced chemokine production, degranulation and NET release, but also reduced motility [4, 5]. Whether these also arise from changes in granulopoiesis, or are consequences of activation of mature neutrophils, and whether these modifications enhance the ability of neutrophils to control parasites, remains to be resolved. However, modifications of neutrophil phenotype which are advantageous to survive malaria, may be disadvantageous when multiple pathogens can infect a host simultaneously or sequentially. The reduced oxidative burst capacity of neutrophils produced during

malaria decreases resistance to invasive *Salmonella* infection, providing a new niche for these intracellular bacteria to replicate [12], and an explanation for the causal association between malaria and non-Typhoid Salmonella bacteremia seen in Africa.

Blame or tame? Neutrophils as therapeutic targets

The emerging evidence that different neutrophil defense mechanisms may contribute to pathogenesis and protection in malaria raises the intriguing possibility that the balance between them might be therapeutically manipulated. To achieve this a more granular understanding of neutrophil behavior and its heterogeneity in malaria will be necessary. Despite the recent insights described above, there are many open questions. Are neutrophil numbers and function important determinants of protection or susceptibility to malaria? What determines the nature (NETs, degranulation, or phagocytosis) and magnitude of the neutrophil response to malaria parasites? Where do these responses predominantly occur - systemically, or in the microvasculature in proximity to sequestered parasites? How do genetic and environmental factors, and previous exposure to malaria, modify the neutrophil response to malaria? To what extent are the neutrophil responses seen in human malaria recapitulated in animal models? Can granulopoiesis be modified to constrain pathogenic neutrophil responses and enhance protective ones? Or is it better to target the neutrophil effectors which mediate harmful or protective responses? It is truly surprising that so much is unknown about the role of most abundant leukocyte population in the circulation in one of the world's most prevalent infections, but it seems like the time is right to start addressing these questions.

Acknowledgments:

We thank Diane Schad for help with illustration.

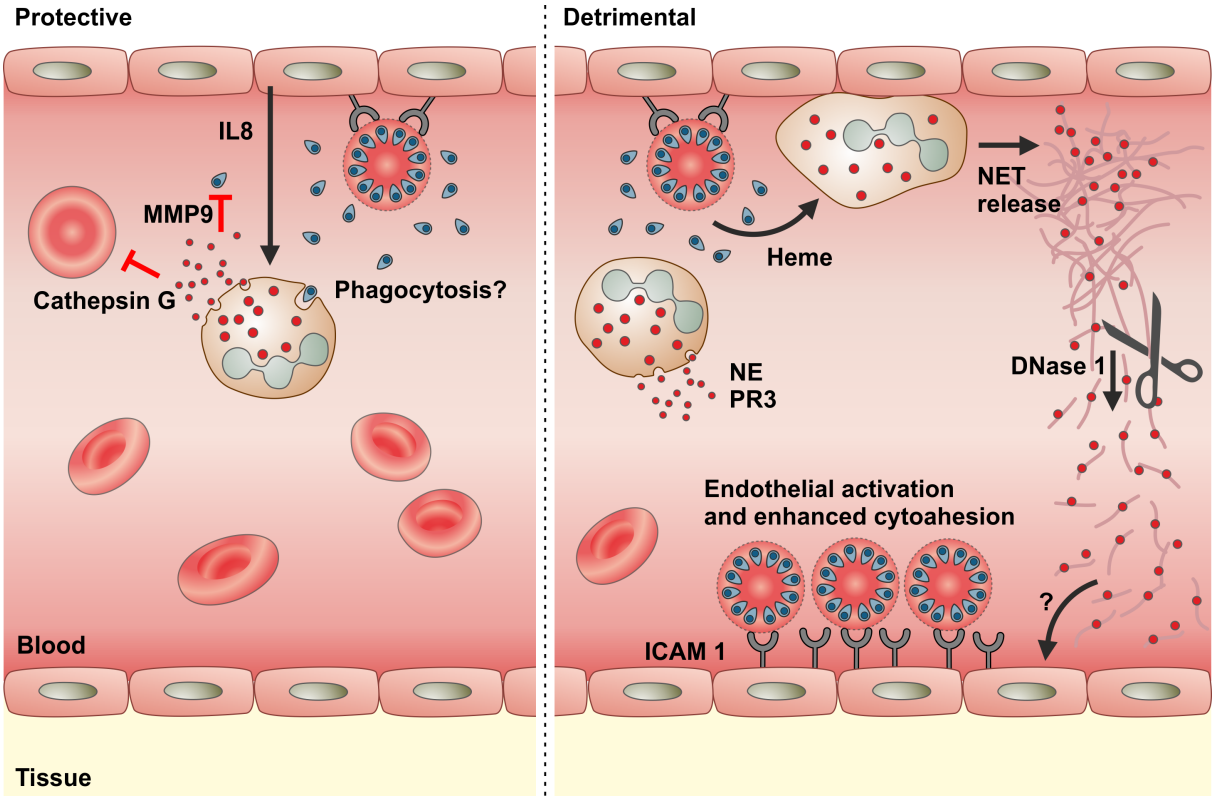
References:

1. Aitken, E.H. et al. (2018) Neutrophils and Malaria. Front Immunol 9, 3005.
2. Knackstedt, S.L. et al. (2019) Neutrophil extracellular traps drive inflammatory pathogenesis in malaria. Sci Immunol 4 (40).
3. Sercundes, M.K. et al. (2016) Targeting Neutrophils to Prevent Malaria-Associated Acute Lung Injury/Acute Respiratory Distress Syndrome in Mice. PLoS Pathog 12 (12), e1006054.
4. Rocha, B.C. et al. (2015) Type I Interferon Transcriptional Signature in Neutrophils and Low-Density Granulocytes Are Associated with Tissue Damage in Malaria. Cell Rep 13 (12), 2829-2841.
5. Feintuch, C.M. et al. (2016) Activated Neutrophils Are Associated with Pediatric Cerebral Malaria Vasculopathy in Malawian Children. MBio 7 (1), e01300-15.
6. Berg, A. et al. (2014) Cytokine network in adults with falciparum Malaria and HIV-1: increased IL-8 and IP-10 levels are associated with disease severity. PLoS One 9 (12), e114480.
7. Lee, H.J. et al. (2018) Integrated pathogen load and dual transcriptome analysis of systemic host-pathogen interactions in severe malaria. Sci Transl Med 10 (447).
8. Georgiadou, A. et al. (2019) Modelling pathogen load dynamics to elucidate mechanistic determinants of host-Plasmodium falciparum interactions. Nat Microbiol 4 (9), 1592-1602.
9. Sun, T. and Chakrabarti, C. (1985) Schizonts, merozoites, and phagocytosis in falciparum malaria. Ann Clin Lab Sci 15 (6), 465-9.
10. Boyle, M.J. et al. (2015) Human antibodies fix complement to inhibit Plasmodium falciparum invasion of erythrocytes and are associated with protection against malaria. Immunity 42 (3), 580-90.
11. Cunnington, A.J. et al. (2012) Prolonged neutrophil dysfunction after Plasmodium falciparum malaria is related to hemolysis and heme oxygenase-1 induction. J Immunol 189 (11), 5336-46.
12. Cunnington, A.J. et al. (2011) Malaria impairs resistance to Salmonella through heme- and heme oxygenase-dependent dysfunctional granulocyte mobilization. Nat Med 18 (1), 120-7.
13. Amulic, B. et al. (2012) Neutrophil function: from mechanisms to disease. Annu Rev Immunol 30, 459-89.

Box 1: A dangerous cargo – neutrophil effector molecules

Neutrophils excel at tracking down and destroying bacteria and fungi. They are the first immune cells that respond to breaches at epithelial and mucosal barriers, where they mobilise their impressive microbicidal armamentarium of proteases, reactive oxygen species (ROS) producing enzymes and antimicrobial proteins. Most of the neutrophils' antimicrobials are stored in granules - preformed cytoplasmic vesicles that can fuse with phagolysosomes containing engulfed pathogens. Granule components can also be released extracellularly by degranulation or formation of neutrophil extracellular traps (NETs) - chromatin-based structures decorated with granule microbicidal molecules, which are expelled via a regulated cell death pathway (Figure I). NETs trap pathogens, prevent their dissemination and contribute to their killing [13].

Figure I. Acute neutrophil responses in malaria Left: Neutrophils are recruited by the chemokine IL-8 and suppress *P. falciparum* replication by phagocytosis of iRBCs and free merozoites and by release of granule proteins. Cathepsin G, released from primary granules, cleaves RBC receptors required for merozoite invasion, while MMP-9, released from secondary granules, has direct antiparasitic activity. Right: severe malaria is associated with excess degranulation of the primary granule proteases neutrophil elastase (NE) and proteinase 3 (PR3), as well as NET release triggered by cell-free heme. NETs are cleaved into fragments by plasma DNAse 1, which leads to endothelial activation via an unknown mechanism. Upregulation of ICAM1 on the activated endothelium enhances sequestration of iRBCs and contributes to pathology. It is currently unknown if NETs are exclusively detrimental in malaria or whether they may also have protective effects at other stages of the disease.


Protective
Detrimental
IL8
MMP9
Cathepsin G
Phagocytosis?
Blood
Tissue
Heme
NET
release
NE
PR3
DNase 1
Endothelial activation
and enhanced cytoahesion
ICAM 1
?

205